Pig vaccines and diagnostics for African swine fever: the case of Uganda

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Key messages

- Rapid diagnostics for African swine fever in outbreak areas and sensitive diagnostics for detecting chronical infected pigs are needed for the control of the disease.
- A vaccine for African swine fever could greatly enhance the control of the disease in sub-Saharan Africa where the large-scale use of strict biosecurity measures are unfeasible.
- Collaboration between International Livestock Research Institute (ILRI) and Friedrich Loeffler Institute, the first of its kind in the field of ASF vaccinology/vaccine development between these organizations, has been very valuable in starting up activities in this area and this work is now followed up in a new project.

Infectious livestock diseases have a huge negative impact on the incomes of resource-poor farmers and the development of national economies. In developing countries, meat consumption has increased by 6% annually over the last five decades and this trend is rising. Pigs are among the most profitable livestock for poor farmers and pig farming is recognized as means of increasing food security and smallholder livelihoods (Costard et al. 2009), particularly of women and young people in sub-Saharan Africa who are disproportionately involved in pig farming due to cultural norms.

However, the viability of pig farming is constantly threatened by viral diseases; one of the most deadly and devastating, African swine fever (ASF) virus, seriously threatens food security and impedes development. Some viral genotypes can cause up to 100%, and less virulent strains between 30–70% mortality. ASF is a very acute disease with death from virulent strains occurring between 6 and 13 days post-infection. From 2009–2011, ASF was reported in 26 African countries (Penrith et al. 2013). When it spread to Ivory Coast and Madagascar, ASF decimated 30–50% of the national pig population (el Hicheri et al. 1998; Roger et al. 2001). Recent outbreaks in the Russian Federation caused losses of approximately USD 1 billion (FAO 2013) and the virus has now spread to other eastern European countries. The epidemiology of ASF is complex. The most efficient transmission is direct contact between pigs, but the disease can also be transmitted indirectly through fomites, through soft ticks and the virus can be maintained in through a wildlife cycle. In endemic areas such as in sub-Saharan Africa, an added risk to the spread of the disease is the underreporting of outbreaks. A vaccine could provide a means of controlling the disease in areas prone to outbreaks and high quality diagnostic tools would prevent outbreak response delays.

African swine fever (ASF) is a haemorrhagic fever affecting pigs. If introduced into a farm, it can kill all the animals within a few days. It is a large DNA virus and the only member of Asfaviridae family, but has similarities with the pox viruses, such as chicken pox and goat pox, among others. The virus is initially found in cells of the macrophage type; but in the later phases of the disease, it also infects other cell types such as epithelial cells. Distinct clinical symptoms are high fever, redness (haemorrhage) of the ears and on other patches of the skin, and death.

Unfortunately, there are no available commercial vaccines or treatment to control or prevent ASF virus infection. The only method of preventing ASF is by using strict biosecurity methods, e.g. fencing of animals, avoiding contact with wild pigs, washing of boots before entering pig enclosures, use of quarantine pens for new animals, etc. Compartmentalization and zoning of pig herds can be used to keep areas ASF-free; however, this approach can be difficult to introduce and maintain on a large-scale in Africa as pigs often roam freely in rural and peri-urban systems.

Moreover, poor African pig producers are less likely to implement control strategies or report disease outbreaks because of a lack of knowledge and incentives to do so. Therefore, this disease poses serious socio-economic consequences in affected and nearby countries and highlights the urgency of developing efficient countermeasures against ASF. With an estimated 34 million pigs in sub-Saharan Africa, an ASF vaccine could benefit from 6–17 million smallholder farmers, providing protection in cases of generalized outbreaks and preventing outbreaks in nearby areas. As the pig population in Africa rises, disease spread could be increasingly problematic.
Towards an African swine fever vaccine

ILRI scientists recently initiated work on the development of an ASF vaccine. In collaboration with the Friedrich Loeffler Institute (FLI), scientists involved in the CGIAR Research program on Livestock and Fish attempted to generate an attenuated vaccine—reducing the virulence of a pathogen, but still keeping it alive, by a method known as di-codon deoptimization. Though the approach has been reportedly successful in slowing down the speed of virus growth (Nouen et al. 2014), it was not so when based on the major capsid protein p72 from the virus. The work is ongoing to broaden the approach to include other genes from the virus.

Instead, two modified viruses were developed based on the deletion of the gene CD2v—a gene involved in the attachment of the virus to the host cells. This was done in two different genotype backgrounds, genotype I and genotype IX. The modified viruses are now ready to be tested in animal experiments. ILRI then employed the necessary laboratory methods for immunological and virological ASF work, including developing an animal model using a Kenyan genotype IX isolate.

Scientists also designed a way of inducing immunity to the same virulent strain by using a sequential immunization schedule with increasing doses. In this way, five pigs treated were rendered immune when subjected to a lethal dose of ASF virus (Riitho et al. in preparation). This is a new way of inducing ASF immunity. It may prove very useful as it has been reported that attenuated viruses sometimes change the genome substantially, as well as the disease pattern and immunological responses (Takamatsu et al. 2013).

Scientists have also sequenced new indigenous pig MHC class I molecules1 (Ilsoe et al. submitted). MHC molecules from the pig bind the T-cell epitopes; this complex is then recognized by the immune cells. The MHC class I molecules have never been defined in indigenous pigs in Africa, and this is important for developing vaccines comprising particular proteins/genes when a cellular response, including T cells is needed. An additional 33 pig samples are currently being sequenced.

Furthermore, efforts are ongoing to improve bioinformatics tools for predicting T-cell epitopes together with University of Copenhagen and Danish Technical University. So far, three swine leucocyte antigen (SLA) molecules have been expressed and produced by University of Copenhagen, and they are in the process of being tested for binding of peptides from a random peptide library. The resultant data will be analysed and used for neural network training for the NetMHCPan program for prediction of CTL epitopes. This will then improve the programs performance on predicting new CTL epitopes in context of SLAs.

African swine fever diagnostics

A number of commercial diagnostic platforms are available for the detection of ASF (Agüero et al. 2003; King et al. 2003; Agüero et al. 2004; Tignon et al. 2011; OIE, 2012; Fernández et al. 2013; Haines et al. 2013), but they have not been widely validated in the African context. Confirmation of ASF outbreaks in Africa can take up to three weeks from the moment symptoms are initially observed and field samples collected to laboratory confirmation of infected pigs. This delay has serious consequences in terms of increasing the risk of the further spread of the disease while diagnosis is being confirmed. Rather than wait for their herds to be slaughtered as a biosecurity measure, farmers tend to sell sick pigs in the market at the first hint of an ASF outbreak.

An ASF rapid diagnostic test, in the form of a pen-side/point-of-care test that is suitable for use in African village-level pig production environments would greatly reduce the diagnosis time lag to less than one hour, greatly reducing associated costs and increasing the efficiency of disease control. To this end scientists have evaluated the use of a rapid diagnostic approach using TETRACORE, a commercial real-time polymerase chain reaction (PCR) (Zsak et al. 2005; LeBlanc et al. 2013). The test is applicable at the point of care and has proved useful in the early confirmation of outbreaks in Kenya and Uganda.

Standardised ASF diagnostic tests tailored for local application are currently lacking (Okoth et al. 2013). Based on recent studies in East Africa, diagnostic platforms addressing various scenarios are needed: epidemiological situations that vary from acute to chronic infections; mixed infections from diseases with ASF-like clinical signs that confound diagnosis; a range of samples available to confirm diagnosis including blood, serum, oral fluids, tissue exudates and internal organs; host characteristics that influence performance e.g. absence of neutralizing antibodies to ASF virus; and virus genotypes including virulent and avirulent strains.

Studies have shown that PCR tests are suitable for early detection of the ASF virus genome during epidemics (Gallardo et al. 2009; 2011). However, specific tests should be matched with their robustness in pigs with low levels of the virus, especially in endemic areas. Due to the limited sensitivity of some ELISA tests in samples with low antibody titres (Okoth et al. 2013), improved serological tests are needed that can detect antibodies within a short period post infection.

Proof-of-concept and standardisation of the diagnostic products and associated assay protocols will inform actors in ASF surveillance, enhancing adoption and application of relevant disease-control technologies in Africa. The target ASF diagnostic products profile and standardized assay protocols suitable for disease surveillance in East Africa are currently being evaluated. This new knowledge on the product profiles for effective ASF diagnostics will result in the appropriate targeting of a panel of diagnostics needed for early detection of the disease in African village-level pig production systems to identify index cases of ASF, followed by rapid response and control.

1 MHC molecules are tissue type antigens (swine leucocyte antigens (SLAs) from pigs) which bind the T cell epitopes.
Conclusions
African swine fever, is a devastating disease which can decimate whole herds of pigs when introduced, impacting negatively on the livelihoods and economy of smallholder farmers in Africa. Recent research advanced has shed light on the immunity to African swine fever virus and on ways of identifying promising vaccine candidates.

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